



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)

Chong Jin Oon)

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Examiner: GOLDBERG, Jeanine Anne

Art Unit: 1634

DECLARATION UNDER 37 C.F.R. §1.132

I, Chong Jin Oon, declare as follows:

1. I am a co-inventor of the patent application identified above and a co-inventor of the subject matters described and claimed therein.

2. I am currently a Consultant Medical Oncologist.

3. From 1982 to 2003 I was the Principal Investigator, and Head, Hepatitis & Liver Cancer Research Unit, Ransome Research Laboratory, Singapore General Hospital, Republic of Singapore.

4. To evaluate HBV strains that have escaped hepatitis B vaccination and/or resist antiviral therapy, we have tested by using PCR for the presence of HBV DNA in samples collected from individuals including those tested negative for HBsAg by EIA following in hepatitis B vaccination or resistant to Lamivudine treatment.

5. Leveraging our many years of experience in studying the HBV genome and mining our proprietary database of HBV variants, we have specifically designed a pair of PCR primers to target the immunogenic major hydrophilic region of HBV. The pair of primers are 5'CAAGGTATGTTGCCCGTTTGT, and 5'TGGCTCAGTTTACTAGTGCCATT, designated as SEQ ID NO: 1 and SEQ ID NO: 2, respectively in the specification of the patent application

HBVDNA at map nucleotide position 418-433 and has a mismatched 5' end which contains a Pst I site; primer HD03 (5' GCGAAGCTTCATCATCCATATAGC) that binds to the complementary strand of HBVDNA at nucleotide position 748-734 and has a mismatch 5' end which contains a Hind III site. Primer MD14 and HD03 were previously described in Oon et al. "Molecular epidemiology of Hepatitis B virus vaccine variants in Singapore" Vaccine (1995), Vol.13, No:8, pp 699-702, and in Harrison T.J. et al. "Independent emergence of a vaccine-induced escape of Hepatitis B mutant of Hepatitis B virus" Journal of Hepatology (1991) 13(Suppl.4), S105-107. These two references were submitted to the Examiner in the IDS mailed March 25, 2003.

6. Generally, to collect samples for the test of hepatitis B surface antigen mutants, individuals were selected based on the following criteria: 1) children who had received standard Hepatitis B Vaccinations; 2) patients with known liver diseases, who had persistent elevated serum SGPT and SGOT levels of over two fold above baseline ($> 50\text{U/L}$) for over six months, HBsAg negative but anti-HBcIgG positive, and negative for HBV DNA (tested by kits from Abbott Labs., North Chicago, USA, and Chiron Corporation, Branched HBVDNA assays), anti-HCV negative, HAVIgM and HEV IgM negative; 3) patients who had developed flare ups of hepatitis B while on Lamivudine treatment, and were HBVDNA positive (Abbott), were collected, and stored for testing; and 4) healthy individuals in random population sampling. The sera of these individuals were collected, separated, coded and stored at -75°C until tested.

7. Specifically, 18 test samples were collected from the following individuals: 1) Negative controls consisted of eight individuals (8) who were HBsAg, Anti-HBcIgG and HBVDNA negative; 2) Seven (7) individuals who were HBsAg negative, HBVDNA negative (Abbott test), anti-HBcIgG positive, but had G145R HBsAg surface mutants; 3) One individual (1) with a mixture of HBsAg surface mutants which were proven on sequencing and cloning analysis to be P120S, G130D, and T131P; 4) One individual (1) with a Lamivudine resistant HBsAg surface antigen mutant, G130D; and 5) One individual (1) with liver disease with a mixture of HBsAg surface mutants, G130D and G145R.

8. The test for the presence of HBV surface antigen mutants were conducted on PCR isolated products by two trained technicians (holders of Diploma in Biotechnology) on the coded samples. Following a clear gel electrophoresis positive identification, the product was sequenced for identification. Any single test which showed a clear positive result on gel electrophoresis and

subsequent sequencing was considered a positive result. Those showing “smear” or “indefinite” were regarded as a negative result.

8. The results of tests using the primer pair of SEQ ID NO: 1 and SEQ ID NO: 2, and those using the primer pair MD 14 and HD03 are compared as follows:

For all 18 tested samples, tests using the primer pair MD 14 and HD03 showed clear non-reactivity, and were negative on the gel electrophoresis.

In the test using the primer pair of SEQ ID NO: 1 and SEQ ID NO: 2, 10/10(100%) PCR products containing various HBV positive surface mutants were identified.


	Numbers Positive <u>MD 14 /HD03</u>	Numbers Positive <u>SEQ ID NO: 1 / SEQ ID NO: 2</u>
1) Negative controls (8)	0	0 (100%)
2) G145R (7)	0	7 (100%)
3) P120S/G130D/T131P (1)	0	1 (100%)
4) G130D (1)	0	1 (100%)
5) G130D/G145R (1)	0	1 (100%)

Amino Acid Symbols: G=Glycine; R=Arginine; P=Proline; S=Serine; T=Threonine.

9. These data show conclusively that the primer pair SEQ ID NO: 1 / SEQ ID NO: 2 were superior to the primer pair MD 14 /HD03. The primer pair MD 14 /HD03 disclosed in the references were unable to detect a variety of HBsAg surface mutants. In contrast, the primer pair SEQ ID NO: 1 / SEQ ID NO: 2 were able to detect various HBV Surface antigen negative, but PCR positive HBV viruses, such as the Glycine145Arginine, Glycine130Aspartate, Proline120Serine, and Threonine131Proline mutants.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By: 
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University, M.D.Cantab, FRCP, London.

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CURRICULUM VITAE

Name : Dr.OON Chong Jin

Qualifications : Master of Arts (Honours), 1964
Cambridge University, England

Batchelor of Medicine, Batchelor of Chirugery(1964)
Cambridge University, England.

Doctor of Medicine(M.D.Cantab.,)
Cambridge University, 1975

Member of the Royal College of Physicians, London
England, 1970.

Fellow of the Royal College of Physicians, London,
England, 1986.

Member of Royal College of Physicians & Surgeons,
London, England, 1965

Diploma in Child Health, Royal College of Physicians,
London, England, 1965.

Fellow of the Academy of Medicine of Singapore, 1976.

Citizenship : Singaporean.

Date of Birth : 11th July, 1939.

Marital Status : Married with two sons.

Religion : Roman Catholic.

Present Appointments :

1. Principal Investigator, Hepatitis & Liver Cancer
Unit Ransome Research Laboratory & Department
Clinical Research,(Ministry of Health, Singapore).
2. Visiting Consultant Physician, Ministry of Health,
Singapore.
3. Member, Scientific & Advisory Committee on
Hepatitis and related disorders, Ministry of Health,

2..

4. Consultant Medical Oncologist,
Mount Elzabeth & Mount Alvernia Hospitals,
Singapore.
5. Consultant to the World Health Organization
for Viral Hepatitis B and Hepatitis B Vaccines,
Global eradication program (15th May, 2002-).
6. Member, World Health Organization Working
Committee on Nucleic Acid Amplification
Technologies. International
Standards and working reagents(National
Institutes of Biological Standards and Control,
Potters Bar, England(16th Jan.2002-)
7. Visiting Scientist, Human Genome Institute,
Singapore.(8th Jan.2003-)

Past Appointments :

Associate Professor of Medicine & Deputy Head,
University Department of Medicine II, Singapore
General Hospital, Singapore(1984-6).

Founding Chairman,
Ministry of Health, Singapore, Scientific &
Advisory Committee on Hepatitis & Related
Disorders (1985-1990)

Consultant , International Agency for Research in
Cancer, World Health Organization,
Hepatitis B Vaccines in the prevention of Liver
Cancer.(1984- 2000)

Consultant and Advisor to World Health
Organization for Liver Cancer, Hepatitis, Hepatitis
B Vaccines, Cancer prevention, and Biological
Standards.(1985- 1990).

3. (a)

Visiting Professor and Scientist to :

From 1985-2003

- Division of Infectious Diseases, Stanford University Medical School(Professor Tom Merigan)
Lecture: Collaboration on Viral Hepatitis b Research.
- Department of Biochemistry , University of Calgary(Prof. Chris Tan).
Lecture: The Hepatitis B Program in Singapore.
- Rockefeller University, New York, USA.(Prof. Chua Nam Hai)
Lecture: Future Biotechnology in Singapore. The setting up of an Institute of Molecular & and Cell Biology in Singapore.(Representative of the Vice Chancellor, and the Singapore Science Council).
- The Liver Unit, King's College Hospital(Prof. Roger Williams)
Lecture: The Hepatitis B Vaccination program in Singapore.
- * Department of Virology, Max Von Pettenkofer Institute(Prof. R. Deinhardt)
Lecture: Prevention and Control of Hepatitis B.
- Royal Free Hospital, London(Professor Sheila Sherlock)
Lecture: Prevention and control of Hepatitis B.
- Balliol College, Oxford and department of Virology, Oxford University.(Professor Baruch Blumberg)
Lecture: New Viruses of Hepatitis B.
- Imperial College of Science and Technology & St Mary's Hospital, London(Professor Howard Thomas)
Lecture: Use of Lymphoblastoid Alpha Interferon in the prevention of Human Hepatocellular Carcinoma..
- Department of Medicine, Hammersmith Hospital Postgraduate Medical School(Professor Karol Sakura)
Lecture: Prevention of Liver Cancer

Curriculum Vitae (Dr. C.J. Oon) Cont'd

Visiting Professor and Scientist to :

- Chiba University Medical School. (Prof. Kunio Okuda)
Discussion and Lecture : Early Diagnosis of Liver Cancer.
- National Taiwan University Medical School. (Prof. Deng Shih-Cheng)
Lecture : The Hepatitis B Vaccination Programme in Singapore.
- Virus Laboratory, Fairfield Hospital, Melbourne, Australia. (Prof. Ian Gust)
- Royal College of Physicians of Edinburgh.
Lecture : Reduction in Hepatitis B following HB Vaccinations.
- Imperial College of Science, Technology and Medicine.
Lecture : Lymphoblastoid Interferon in prevention of liver cancer in high-risk HBV carriers. St. Mary's Hospital, London. (Prof. Howard Thomas).
- Dept. of Medicine, Queen Mary Hospital, Hong Kong. (Prof. David Todd)
Lecture : New Viruses of Hepatitis B.
- Beijing Cancer Hospital, China. (Prof. Yan Sun)
Lecture : Liver Cancer, Prevention and Early Treatment.
- Dept. of Virology, Hangzhou, China. (Prof. Liu Ke-Zhou)
Lecture : Liver Cancer, Prevention and Early Treatment.
Liver Unit, St. Mary's Hospital. (Dr. Graham Foster)
- Institut d'Virologie, Tours, France. (Prof. Pierre Tiollais)
Lecture : HB Vaccine.
Institute Pasteur, Paris, France. (Prof. Pierre Tiollais).

Awards, Nominations, Honours, Scholarships

- 5th Nov 1997 : Distinguished Phone-Poulenc Oration - Hepatitis B, Past, Present and the Future. Indian National Association for the Study of the Liver, Hyderabad, India.
- 7th Sept 1995 : Ministry of Health's nomination for National Science and Technology Award.
- 5th Sept 1993 : Ministry of Health's nomination for National Science Award.
- June 1975 : French Government & National University of Singapore Scholarship to France for the Study of Immunology of Leukaemia.

Others

- 1978 - 1982 Founder Secretary General, Asian Pacific Association for the Study of the Liver
- 1983 - 1986 Consultant, Singapore Biotech Pte Ltd
(Singapore Company, responsible for the Industrial Manufacture of the Hepatitis B Vaccine for the ASEAN INDUSTRIAL PROJECT).
- Government's Principal Scientist
Hepatitis B Vaccine Project (for ASEAN INDUSTRIAL PROJECT)
and negotiation teams with Taiwan, ASEAN and the People's Republic of China.
- Member, Biotechnology Programme,
Economic Development Board and
National University of Singapore
- 1985 Member, Cancer Control Work Group
Ministry of Health, Singapore

Currently also, Member, International Association for the Study of the Liver.

Past Employment

- 1970 - 1975 Lawson Cancer Research Fellow
Physician-in-charge, Tumour Biology Group
Westminster Hospital, London S.W.I
- Hon. Senior Registrar
Westminster Hospital, London S.W.I
- Lecturer in Clinical Oncology
Westminster Hospital, London

Research Interests/Publications

Published extensively on the epidemiology of hepatitis, hepatitis B vaccine strategies, including low dose vaccines for endemic regions in low risk groups, treatment of primary liver cancer and strategies of prevention of liver cancer, antiviral strategies for chronic hepatitis B, variants of hepatitis B and hepatitis C (over 200 publications since 1975) and public health control of viral hepatitis infections.

Travelling Fellowships

Visiting Professor to Stanford University Medical School, Stanford, Palo Alto, U.S.A, Rockefeller Institute (Biotechnology), Department of Biochemistry, University of Calgary, Canada and Max Planck Institute of Medical Virology, Munich, Germany (1984)

Publications

1. Oon CJ, Geddes AM, Bridgewater FA, Williams DN, Grimshaw GJ.
Clinical and bacteriological studies with Clindamycin, British Medical Journal 20 June 1970, 2 : 703-4.
2. Oon CJ, Elliott P, Buckleton Hentietta, Wright J, Cooke KB, Apsey M, Whitsed H.
The invitro response of anti melanoma antibody conjugated to Chlorambucil on short term human melanoma cell lines. Revue de l' Institut Pasteur de Lyon. 1973 t6, No. 3, (pp 195 a 199).
3. Oon CJ, Malka S, Hobbs JR. Increase in IgD bearing lymphocytes in patients with malignant melanoma. J. Cancer 1974, 30, 379.
4. Oon CJ, Hobbs JR. Medical problems in donors on treatment using the Continuous Flow Blood Separator. Proc. Int. Symp. Leucocyte Separation and Transfusion (London) 1974.
5. Oon CJ, Apsey M, Buckleton H, Cooke HB, Hanham I, Hazarika P, Hobbs JR, McLeod B. Behring Int. Mitt, Research Communications 1974, No. 56, 228. Report on the proceedings of the International Symposium on Melanoma Antigens.
6. Oon CJ, Butterworth C, Apsey M, Elliott P, Rossengurk N, Hobbs JR. Homologous Immunotherapy using immune leucocytes and Chlorambucil treated tumour specific antibodies melanoma and neuroblastoma. Proc. Inter. Symp. on Leucocyte Separation and Transfusion (London). 1974, Abst. G.5.
7. Oon CJ, Westbury G, Butterworth C, Elliott P, Hobbs JR. Homologous immunotherapy with immune leucocytes in human cancers. Behring Instit. Mitt, Research Communication 1974, No. 56, 346.
8. Oon CJ, Butterworth C, Westbury G, Hobbs JR, Europ J. T-lympocyte responses in patients with Malignant Melanoma. Cancer 1974, Vol. 10. pp 636-646.
9. Oon CJ, Hobbs JR. Clinical Applications of the cell separator. Clin. Exp. Immun. 1975, Vol. 20, 1-6.

10. **Oon** CJ, Tan RSH, Barrett JR. Leukaphoresis as treatment for the Sezary Syndrome. *Proc. Roy. Soc. Med.* 1975, 68, 648.
11. **Oon** CJ, Faroozanfar N, Hobbs JR. Progbhase deficiency in identical twins with chronic granulomatous osteomyelitis corrected by fresh plasma transfusion. M.D. Thesis, 1975, p. 185-193, Part III.
12. **Oon** CJ. The clinical applications of the Continuous Flow Blood Separator Machine. M.D. Thesis, Cambridge University 1975.
13. **Oon** CJ, Hobbs JR. Medical problems in Donors on treatment using the Continuous Flow Blood Separator. In : *Leucocytes Separation, Collection and Transfusion*. Edited : J.M. Goldman, R.M. Lowenthal. Academy Press, London, New York, San Francisco, 1975.
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15. **Oon** CJ, Hobbs JR. The use of human gamma globulin treated with cytotoxic drugs for treatment of human cancers. *Proc. 2nd Asian Cancer Congress*, 1976, Ed. Shanmugaratnam et al. 358-363.
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17. **Oon** CJ, Chua D, Yo SL. Alphafoetoprotein studies in Liver Diseases. *Proceedings of the 5th Asia Pacific Congress in Gastroenterology*. May 24-27, 1976, Singapore (Ed. S.K. Lee et al), 477
18. **Law** CH, Tan AYO, Oon CJ, Chen F, Ho KT, Leong SF, Ng PL, Simons MJ. Cellular Expression of HBsAg in Liver Diseases. *Proceedings of the 5th Asia Pacific Congress of Gastroenterology*, May 24-27, 1976, 290-293.
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33. Tan WC, Yo SL, Chua D, Cheah JS, Oon CJ. Raised serum L-thyroxine in patients with Hepatocellular Carcinoma. Annals Academy of Medicine, April 1978, Vol. 7, No. 2, 129-134.

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35. **Oon** Chong Jin. The treatment of Primary Hepatocellular Carcinoma. In : Proc. Asian Cancer Conference, Manila, 1978.
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38. **Oon** CJ, Yo SL. Presence of a Hepatoma-Liver Antigen in the sera of patients with Primary Hepatocellular Carcinoma. Singapore Medical Journal, 1979, Vol. 20, 317-322.
39. **Oon** CJ, Chio LF. Changes in of serum Alpha₁antitrypsin, alpha₁acid glycoprotein and Beta₂ glycoprotein I in patients with malignant hepatocellular carcinoma. Cancer 1979, Vol. 43, No. 2, 596-604.
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42. **Oon** CJ, Okuda K, Pang R. Report of the Research Subcommittee (Asian Pacific Association for the Study of the Liver) on Primary Liver Cancer and Chronic Hepatitis in Asian Pacific Countries. World Health Organisation, WP C2, 81/1. 16 Nov. 1979 (Endo) 1-79.
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44. **Chan** SH, Simons MJ, **Oon** CJ. Histocompatibility antigens and Hepatocellular Carcinoma. J. Nat. Cancer Institute, July 1980, Vol. 65, No. 1, 21-23.

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46. **Oon** CJ. Hepatitis HBV virus and alphafoetoprotein in Liver Diseases in Singapore. *Singapore Medical Journal*, 1980, Vol. 21, No. 2, 506-508.
47. **Chan** SH, Heng SH, Yo SL, Oon CJ. Comparison of various assays for detection of AFP in differentiating patients with primary hepatocellular carcinoma from controls. *J. Clin. Path.* 1980, Vol. 33, 792-793.
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49. **Oon** CJ, Yo SL, Chio LF, Chua D. The evaluation of tumour marker proteins in the diagnosis of Primary Hepatocellular Carcinoma. *Ibid.*, 228-233.
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51. **Oon** CJ, Chua EJ, Foong WC, Tan LKA, Chiang CH, Ho ST, Seah CS. Adriamycin in the treatment of resistible and irresistible primary hepatocellular carcinoma. *Ibid.*, 256-259.
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53. **Oon** CJ, Lee YS. Medullary infarction - was it Depoprovera? *Singapore Medical Journal*, 1980, Vol. 21, No. 5, 717-719.
54. **Oon** CJ. Multiple tumour markers in the diagnosis of Primary Hepatocellular Carcinoma. *Therapy of Hepatobiliary Diseases* (Ed. Prof. Yieh Wei Fa, China).
55. **Oon** CJ. Review - Current States of medical treatment for Primary Hepatocellular Carcinoma. *Proc. International Congress of Upper Gastrointestinal tumours. International Congress Series No. 542, Mainz Sept. 9-11, 1980.* Ed. M. Friedman, M. Ogawa, D. Kisner, Excerpta Medica, Amsterdam, Oxford, Princeton.

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57. **Oon** CJ. Cancer Research in Singapore. Singapore Science Bulletin Report. MC(P) 93/1/81, No. 1, 1981, 2-3.
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59. **Oon** CJ. Leukaemia a Cell Biology, Immunotherapy and Bone Marrow Transplantation. New concepts on the Acute Leukaemias : Morphology, Classification, Cell Biology, Chemotherapy, Supportive Care, Immunotherapy and Bone Marrow Transplantation. Annals Acad. Medicine 1981, Vol. 10, No. 3 353-363.
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64. **Oon** CJ. Oncology - The past, the present and the future in Singapore. Annals Academy of Medicine, Singapore, 1982, Vol. 11, No. 3, 393-397.
65. Chong-Jin **Oon**, Michael A. Friedman. Primary Hepatocellular Carcinoma. Present state of the Disease and Prospects for the Future. Cancer Chemother. Pharmacol, 1982, 8 : 231-235.
66. **Oon** CJ, Lim KP, Singh R. Hepatitis B Infection in Hepatocellular Carcinoma and other malignancies. Abstract 86, Hepatology, 1982, Vol. 2 No. 1:133.

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72. Quak SH, **Oon** CJ, Singh R, Wong HB. Hepatitis B Markers in Singapore - A cross Sectional Study of 431 children. Annals of Tropical Pediatrics, 1982, 2, 53-56.
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74. **Oon** Chong Jin. Tumour markers protein in the diagnosis of Hepatocellular Carcinoma. In : Advances in Digestive System Diseases. Editor : Ye Wei Fa, 1982. People's Republic of China.
75. **Oon** Chong Jin. Immune Status of various population to HBV in Singapore and a Strategy for its prevention and immunoprophylaxis. In : Developments in Biological Standardisation. Viral Hepatitis Standardisation in Immunoprophylaxis of Infection by Hepatitis Viruses, 1982, Vol. 54. S. Karger, Basel, Munchon, Paris, London, New York.
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